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The history and current status of Extended Spectrum Beta Lactamase producing bacteria in Sweden

A literature review on Extended Spectrum Beta Lactamase producing bacteria and its history and current status in Sweden with emphasis on food producing animals and pets.

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Abstract

Bacteria with the Extended Spectrum Beta Lactamase (ESBL) resistance mechanism have become more common in Sweden in the last couple of years. Sweden has worked to diminish and control antimicrobial resistance for a long time, and although Sweden has a favourable situation from an international standpoint, we do see increased yearly cases of the ESBL producing bacteria in human healthcare and, in some instances, in veterinary care as well. Although not commonly found, ESBL producing bacteria have been discovered in Swedish cattle and pigs in low amounts and in imported samples of beef and pork. The occurrence of ESBL producing bacteria in Swedish broilers was high in the last decade and has reached low levels only recently. Companion animals like cats and dogs has also had low levels of ESBL producing bacteria in Sweden, but risk factors, such as feeding commercial raw diets to dogs, have been identified. Horses, and particularly mares in stud farms, have been shown at carry ESBL producing bacteria, but when screenings of ESBL producers in horses from stud farms and horses arriving to equine hospitals were performed, no alarmingly high occurrence was found. ESBL producing bacteria in animals are important, partly due to the risk of animals providing as reservoirs and breeding grounds for these bacteria and partly due to the risk of transference to humans.

Contents

1. Introduction

The development of antimicrobial drugs and thus the ability to treat microbial infections has been one of the most important events in the history of modern medicine. Since the development of antimicrobial drugs in the early 1900s antimicrobial drug use has led to resistance, which has become a major global health issue since it results in infections with higher mortality, morbidity, and lengthier treatments. Thus, higher costs are also associated with the health care of these infections (Medina & Pieper, 2016). In the most recent decades, the rise of multi-resistant bacteria has accelerated (Mancuso, et al., 2021). The bacteria have developed mechanisms to inhibit the functions of the antibiotic drugs and one example of such resistance mechanism is Extended Spectrum Beta Lactamase (ESBL) production. Beta lactamase is an enzyme produced by certain bacteria, able to hydrolyse the structure of beta lactam antibiotics and ESBLs are beta lactamases with the ability to hydrolyse the structure of Extended Spectrum Cephalosporins (Livermore, 2008), which are newer generations of an antibiotic class listed as CIA (Critically Important Antimicrobials) by the World Health Organization (World Health Organization (WHO), 2019). Historically, Sweden has had a good status compared to other countries when it comes to antimicrobial resistance. This is hypothesized to be due to effective strategies in limiting the spread of resistance and a promotion for responsible antibiotic usage (Wierup, et al., 2021). The Swedish National Veterinary Institution (SVA) and the Public Health Agency of Sweden cooperate on SWEDRES-SVARM which is a surveillance programme that writes annual reports on antimicrobial resistance and antibiotic sales in Sweden from the veterinary and human healthcare sectors since 2012 (SVARM is the Swedish Veterinary Antibiotic Resistance Monitoring programme that has been ongoing since 2000). And although Sweden does have a favourable situation from an international perspective, we do see an increase in antimicrobial resistance (EFSA Panel of Biological Hazards (BIOHAZ), 2011; Public Health Agency of Sweden; National Veterinary Institute, 2022). This is a worrying trend, and the situation requires close monitoring and a serious approach. The ESBL producing bacteria are not exclusive to humans nor animals and thus, the transference of ESBL producing bacteria between humans and animals, should be taken into consideration (Public Health Agency of Sweden; National Veterinary Institute, 2022; van den Bunt, et al., 2020; Börjesson, et al., 2016). The threat of ESBL producing bacteria highlights the need for strict biosecurity and hygiene when dealing with production animals and their products. In Sweden, the existence of ESBL-producing bacteria in animals have been limited to a low to moderate level thus far. $ESBL_{CARBA}$, which are a class of ESBL enzymes that confers resistance to carbapenem antibiotics as well as to extended spectrum cephalosporins (Giske, et al., 2009), has never been detected in animals in Sweden. However, the occurrence of ESBL and $ESBL_{CARBA}$ producing bacteria in humans is greater and increasing (Public Health Agency of Sweden; National Veterinary Institute, 2022). The objective of this review is to present and summarize the current knowledge of the presence of ESBL-producing bacteria in Sweden and draw conclusions on the trends and history.

2. Materials and Methods.

The material reviewed in this study stems largely from the SVARM reports from the years 2000 to 2011 and the combined SWEDRES-SVARM reports from 2012 to 2022. SVARM reports are Swedish Veterinary Antibiotic Resistance Monitoring reports and SWEDRES is the human medicine equivalent, Sweden Antibiotic Sales and Resistance in Human Medicine. I used these reports for their antibiotic resistance data and for their various summaries of current topics within the ESBL research. SVARM has been published by the SVA since 2000. Since 2012, both reports are published as one by the Public Health Agency of Sweden and the SVA. I used the study of indicator bacteria within the SVARM report which have used isolates of *Escherichia coli* (*E. coli*) and in the reports earlier than and including 2014, *Enterococcus faecalis* and *Enterococcus faecium* isolates were also used as indicator bacteria. I also used the report's data on clinical isolates taken from dogs, cats, horses and in some cases pigs and cattle.

I used the Google Scholar search engine with the search words: "Antimicrobial resistance in Sweden", "ESBL definition", "ESBL in Sweden"," ESBL in Swedish broilers", "ESBL in Swedish horses", "ESBL in horses", "ESBL in dogs", "ESBL in Swedish dogs", "ESBL in food producing animals in Sweden", "ESBL zoonotic Sweden", "ESBL i Sverige", "ESBL hos produktionsdjur", "ESBL plasmid transference", "antimicrobial treatment in horses" and "colistin resistance in Swedish horses".

I gathered information from the webpage of the Public Health Agency of Sweden (www.folkhalsomyndigheten.se), the European Centre for Disease Prevention and Control webpage (www.edcd.europa.eu), the European Food Safety Authority (EFSA) webpage (www.efsa.europa.eu) and the SVA webpage (www.sva.se).

To define what type of resistance that should be included in the review, I used the definition of Giske et al (2009). This is also the definition used in the SVARM/SWEDRES-SVARM reports. The definition of Giske et al (2009) encompasses a broad variety of ESBL-types: "classic" types that are inhibited by beta lactamase inhibitors, "miscellaneous" types which includes the plasmid mediated AmpC enzymes and the ESBL-CARBA which are enzymes that are also resistant to carbapenem antibiotics. Any resistance to extended spectrum cephalosporins caused by chromosomal hyperproduction of AmpC or other mechanisms are therefore not included.

3. Results and Discussion

3.1 Sweden's actions against antimicrobial resistance

Since antibiotics were introduced to the veterinary practice in the 1950s, careful usage of these drugs has been discussed in the Swedish veterinary community and the risks of antibiotic resistance have been a frequent topic in these discussions (Wierup, et al., 2021). From an international perspective, Sweden has for a long time had a favourable situation when it comes to antibiotic usage and resistance (Public Health Agency of Sweden; National Veterinary Institute, 2022). This might be in part, due to the emergence of methicillin resistant *Staphylococcus aureus* (MRSA) and penicillin resistant *Streptococcus pneumoniae* in the 1990s when extensive action plans to prevent further development of these issues were created. These action plans led to close collaborations between researchers and governmental bodies. Notably, in the case of the monopolized pharmacy which provided data on antibiotic sales with almost 100% national coverage (Struwe, 2008). Amongst many actions taken, volunteer regional groups of multisectoral collaboration called STRAMA (Swedish Strategic Programme Against Antibiotic Resistance) started operating in 1995 (Strama Historik, 2023). In

2006, the government passed a bill on the prevention of antibiotic resistance and made STRAMA a government commissioned operation (Struwe, 2008). As another step towards closer surveillance of antimicrobial resistance and to gather information on antimicrobial resistance among animals on a national level SVARM, the Swedish Veterinary Antimicrobial Resistance Monitoring programme was created in 2000. Since then they have published yearly reports on the antimicrobial susceptibility of certain zoonotic bacteria, specific animal pathogens including commensal bacteria like *E. coli* and some *Enterococcus* species. Thus, this early response to antibiotic resistance at the end of last century and in the early 2000s raised a public awareness of the issue that in part contributed to our favourable situation today (Wierup, et al., 2021). The control of antimicrobial resistance is made easier by close collaboration between the government, the veterinary industry, the farmers, and the industries connected to animal production (Struwe, 2008). The Federation of Swedish Farmers (LRF) acts as such a bridge between the government, the farmer industries, and the farmers interests. They are helpful in dispersing information to farmers, including increasing the awareness of antibiotic resistance and usage in the farming communities (Lantbrukarnas Riksförbund, 2023). This cooperation between stakeholders, the early recognition and awareness of antibiotic resistance in the 1900s and the monitoring programmes have facilitated a low use of antibiotics in Sweden due to successful long-term efforts to prevent and control infectious diseases (Struwe, 2008).

3.2 Defining ESBL and ESBL producing bacteria.

The term ESBL was first used in 1989. It then described the enzymes mutated from the broad-spectrum beta lactamases that were able to hydrolyse the extended spectrum cephalosporins and monobactams (Bush, 2008). Since its emergence, the term has begun to include various mutations of the original types of ESBLs as well as enzymes exhibiting near ESBL activity and enzymes capable of hydrolysing carbapenems. There is currently no agreement on the definition of ESBL, although several definitions have been suggested (Livermore, 2008).

Giske et al (2009) concluded that the complex nature of the term ESBL was causing issues for healthcare professionals thus, they proposed a classification scheme of these enzymes that would be more clinically relevant. They stressed that this classification scheme is not meant to replace the more detailed classifications created by the beta-lactamase research but rather to complement it and make the term more useful to healthcare professionals.

The first class according to this definition is the $ESBL_A$. This class includes the enzymes that are not susceptible to extended spectrum cephalosporins but are inhibited by beta lactamase inhibitors. The second class, $ESBL_M$ includes miscellaneous ESBLs with resistance to extended spectrum cephalosporins and specific phenotypes or genotypes, including plasmid-mediated AmpC. The last class is $ESBl_{CARBA}$. These are enzymes with resistance to extended spectrum cephalosporins that also exhibits resistance to at least one carbapenem antibiotic (Giske, et al., 2009). This is the definition I have used in this literature review paper. I have included bacteria with resistance to extended spectrum cephalosporins antibiotics according to these various types of ESBLs and excluded the bacteria exhibiting resistance to extended spectrum cephalosporins through chromosomal hyperproduction of AmpC.

3.3 ESBL producing bacteria in pigs and cattle.

ESBL producing *E. coli* has been specifically monitored in healthy animals by SVARM since 2008 when they found that the occurrence of ESBL producing *E. coli* in pigs was, at its height, rare. In 2008, caecal content of healthy pigs at slaughter was sampled and tested for resistance. The majority, 79% of 349 isolates, were susceptible to all 12 antibiotics tested. Although resistance to extended spectrum cephalosporins was occasionally detected, no ESBL production was found in any of the bacterial isolates tested (National Veterinary Institute (SVA), 2008). This was true throughout the SVARM reports screening pigs until the first detection of ESBL producing *E. coli* in Swedish pigs occurred in 2011 when 1.6% of the samples of intestinal content taken from healthy pigs were tested positive of ESBL production (Swedish National Veterinary Institute (SVA), 2011). This low prevalence trend has continued in the Swedish pork industry until today. The highest occurrence of ESBL producing *E. coli* was seen in 2017 when 4% of the 241 samples taken were positive for ESBL production (Public Health Agency of Sweden;National Veterinary Institute (SVA), 2017). Resistance to Extended Spectrum Cephalosporins caused by other resistance mechanisms than ESBL has been detected more readily throughout the SVARM reports in pigs and other animals. Mostly we find the AmpC-beta lactamase that is causing resistance by chromosomal hyperproduction, as opposed to the plasmid-mediated AmpC belonging to the $ESBL_M$ class (National Veterinary Institute (SVA), 2008; Public Health Agency of Sweden; National Veterinary Institute, 2022).

In the case of cattle, a similar trend of low ESBL occurrence is seen. Low selection pressure amongst cattle older than 6 months was demonstrated in the SVARM report of 2009 when fattening calves were sampled and 95% of the 223 isolated were susceptible to all 12 antimicrobials tested. *E. coli* resistant to extended spectrum cephalosporins were found but none produced ESBL. However, as demonstrated in the SVARM report of 2006, multiresistance, meaning the resistance to three or more antibiotic classes (Swedish National Veterinary Institute (SVA), 2011; National Veterinary Institute (SVA), 2006), of *E. coli* in calves younger than 6 months is more common. This suggests that besides the factors of selection pressure, the occurrence of resistant *E. coli* strains might also depend on age (National Veterinary Institute (SVA), 2009). The results of a research project using similar methods as SVARM were included in the SVARM-SWEDRES report of 2012 and it showed the first finding of ESBL-producing *E. coli* in dairy calves. 742 calves from 249 farms were sampled by rectal swabs. ESBL producing *E. coli* was detected in 9 (1%) of the samples (National Veterinary Institute; Swedish Institute for Communicable Disease Control., 2012). Although this was the first finding of ESBL producing bacteria in Swedish dairy calves the occurrence was low even though a substantial amount of calves were sampled. The following year, in 2013 there was one finding of ESBL-producing Enterobacteriaceae in healthy calves and the low occurrence therefore continued its' trend. In the SWEDRES-SVARM 2021 report an unusually high percentage of ESBL producers was found in 57 sampled cattle under one year of age when 12% of these isolates carried ESBL genes (Public Health Agency of Sweden; National Veterinary Institute, 2021). Notably, five of these isolates were taken from animals slaughtered at the same slaughterhouse on two separate days in 2020 and four of the isolates were closely related. That means that this higher proportion of ESBL producing *E. coli* should be assessed with caution since the isolates came from a limited set of samples and were not from a representative portion of the Swedish cattle population. In conclusion, ESBL producing bacteria is not commonly found in either pigs or cattle and when found, only a few percentages of the samples carry it. ESBL producing bacteria in any amount are unwanted due to the risks associated with them, and though this low occurrence that we see in Swedish pigs and cattle is not significant in terms of numbers, the overall presence of ESBL producing bacteria in our food chain is worrisome.

3.4 ESBL producing bacteria in broilers.

In the 2010 SVARM report, ESBL was recognized as an emerging and severe challenge in the human healthcare (National Veterinary Institute (SVA), 2010). The occurrence of ESBL producing bacteria had been increasing in both human and animal healthcare during this time (Smet, et al., 2010). No resistance to extended spectrum cephalosporins was found in broilers in the earlier years of SVARM. Three isolates were found to be resistant to extended spectrum cephalosporins but not ESBL producing in 2007 but in SVARM 2010 the three isolates taken in 2007 were retrospectively confirmed as ESBL producers (National Veterinary Institute (SVA), 2010). The SVARM report of 2008 and 2009 focused their indicator bacteria investigations on populations of pigs and sheep in 2008 and fattening calves in 2009 and broilers were not investigated during those years (National Veterinary Institute (SVA), 2008; National Veterinary Institute (SVA), 2009). In 2010 broiler and horse populations were sampled for indicator bacteria and ESBL producers were found in both species. The most surprising, and alarming, results were found in broilers when 200 caecal content samples from broilers were taken from seven slaughterhouses processing a total of 99% of Sweden's broilers. A surprising 34% of them contained ESBL producing *E. coli*. This was a confusing result since cephalosporin antibiotics are not used in the Swedish broiler industry and therefore this occurrence could not be explained by selection pressure caused by antibiotic usage within the industry (National Veterinary Institute (SVA), 2010).

These findings in 2010 prompted a collaborative project between the SVA, the National Food Agency and the Swedish Institute for Communicable Disease Control. The project showed that a significant amount of both imported and domestic broiler meat carried ESBL producing bacteria (Nilsson, et al., 2014). The carriage in imported broiler meat was highest in the South American imports where 95% of samples contained ESBL producing *E. coli*. Imported broiler meat of European origin had a carriage rate of 61% while 44% of Swedish broiler meat and 15% of Danish broiler meat were confirmed to contain ESBL producing *E. coli* (Börjesson, et al., 2016). Neither Sweden nor Denmark uses cephalosporins in their broiler productions so again, the relatively high occurrences of ESBL producing *E. coli* in these meat samples could not be linked to a high selection pressure within the production. A study on the situation hypothesised that the occurrence of these bacteria in the Swedish broilers was largely due to imported breeding stock. This hypothesis was supported by results from the study showing that one clone of ESBL producing *E. coli* was found throughout the production pyramid and in flocks of imported grandparent stock (Nilsson, et al., 2014). In the 2011 SVARM report 100 samples were taken from broilers and screened for ESBL. 54% of the samples has *E. coli* positive for ESBL production and this was the highest percentage of ESBL producing bacteria found in Swedish broilers (Swedish National Veterinary Institute (SVA), 2011). Numbers continued to be high in 2012 when a wider spectrum of the Swedish poultry industry was sampled and not only 200 broilers samples but also 69 layer-hen samples and 97 samples from broiler meat were taken. The occurrence of ESBL producing *E. coli* was shown in the layers (13% of samples) and in the broiler meat (41% of the samples). 49% of the 200 broilers samples were positive for ESBL producing *E. coli* and this is a slight decline from the previous year (National Veterinary Institute; Swedish Institute for Communicable Disease Control., 2012). The occurrence of ESBL producing bacteria in poultry was continuously monitored since the surprising finding in 2010 and since then the numbers have been decreasing with the exception of 2015 when the percentage of samples containing ESBL producers was 39% compared to 36% in 2014 (Publich Health Agency of Sweden; National Veterinary Institute (SVA), 2015). But the following years we saw a drastic decrease in ESBL producing bacteria found in Swedish broilers and broiler meat (*see figure 1.*). The SVARM report of 2022 sampled 305 broilers with a result of a 2% carriage of ESBL producing *E. coli* which was a significant decrease from the outbreak in 2010. The difference in methodology spanning the years 2010-2022 should be considered, an exact comparison of the number of ESBL producing *E. coli* found in broilers between the years 2010 and 2022 cannot be made due to two different methodologies begin used. Although, some comparison is warranted since the samples of 2015, the first half of 2016 and 2021 were tested with both methods (Public Health Agency of Sweden; National Veterinary Institute, 2022).

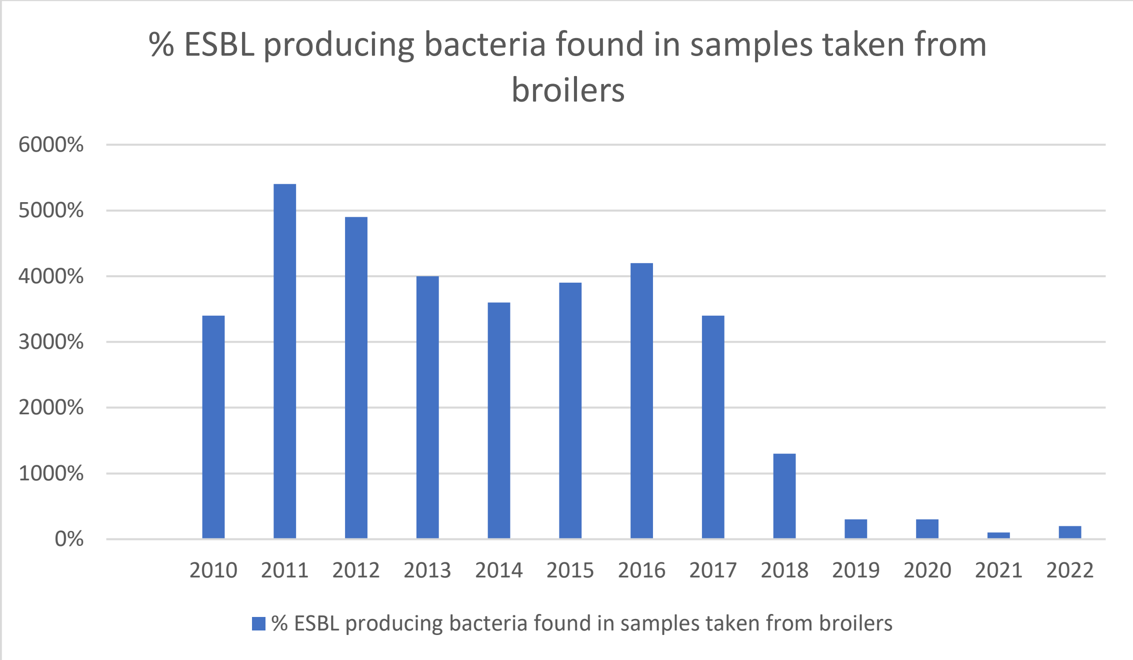

Figure 1. The percentages of samples found positive for ESBL production in the SVARM reports from 2010 to 2022.

3.5 ESBL producing bacteria in dogs, cats, and horses.

No active surveillance exists for ESBL producing bacteria in companion animals and horses as it does for farm animals. Clinical isolates from companion animals and horses have been investigated within the SVARM project since its' early years but from 2010 and onwards, the veterinary laboratories have been encouraged by SVA to submit samples suspected of ESBL producing *Enterobacteriaceae* for verification and genotyping. Since 2017 genome sequencing has been performed on all ESBL producing isolates submitted to SVA. In addition to this, since a few years back the investigation of clinical isolates with extended spectrum cephalosporin resistance has been more readily done since it's free of charge at the laboratories to test for ESBL production in samples with extended spectrum cephalosporins resistance. It must be mentioned, however, that clinical isolates are not considered to be an accurate reflection of the population since they are provided by sick individuals. These individuals may have a higher likelihood of being treated with antibiotics and thus encouraging resistance through selection and, these clinical isolates may represent a group of more resistant bacteria since they are more likely to have caused disease in an individual and a sample has been submitted to identify the pathogen (Publich Health Agency of Sweden; National Veterinary Institute (SVA), 2015).

Bacteria producing ESBL continues to be rare also in Swedish companion animals and horses. A recent Swedish study on ESBL producing Enterobacteriaceae in healthy dogs from 2020 shows that only 3 out of 325 screened dogs carried it (Börjesson, et al., 2020). ESBL in cats has been held at low levels during the years of testing clinical isolates with a height of 8 cats being confirmed to carry ESBL producing bacteria in 2020 (Public Health Agency of Sweden; National Veterinary Institute (SVA), 2020). ESBL producing bacteria were found in Swedish stud-farms around the year of 2010 and in recent years, clinical isolates from companion animals and horses submitted for ESBL screening to the SVA have increased. This is thought to be due to both increased awareness among veterinary clinicians and because funding from the Swedish Board of Agriculture has made it possible to offer ESBL confirmation for free (Publich Health Agency of Sweden; National Veterinary Institute (SVA), 2015).

ESBL producing Enterobacteriaceae started to emerge in Swedish dogs in the early 2000s. In 2008, one diagnostic submission from a dog was confirmed to carry ESBL producing bacteria (National Veterinary Institute (SVA), 2008). In 2013 that number had increased to 14 and in the SVARM report of 2022 it was 15 (Public Health Agency of Sweden; National Veterinary Institute (SVA), 2013; Public Health Agency of Sweden; National Veterinary Institute, 2022). This is no major increase and considering that the testing became free of charge around this time, it might not be an increase in the ESBL producing bacteria as much as an increase in submitted samples due to increased awareness as well as a more readily available tests. The study done on ESBL producing *Enterobacteriaceae* in healthy dogs in 2020 concluded that the existence of these bacteria in Swedish companion dogs was low (Börjesson, et al., 2020). The study cooperated with 8 of the AniCura animal hospitals in Sweden to sample healthy dogs scheduled for such veterinary appointments like vaccinations, blood donations or hip x-rays. Rectal swabs were taken from 325 of these dogs over the age of one year old. Three of the sampled dogs were confirmed to carry ESBL producing *E. coli*. A result of 3 ESBL producing samples out of 325 suggests that ESBL producing *Enterobacteriaceae* does not indicate a common occurrence of such bacteria in healthy dogs in Sweden. However, the study mentions that had any of those ESBL producers been the cause of disease in these dogs, the treatment options within the current

Swedish legislation for antibiotic use in small animals would have been extremely limited, possibly even non-existent (Börjesson, et al., 2020). Therefore, even though the number of ESBL producing bacteria in Swedish companion dogs are not high at the moment, the possibility of these bacteria causing disease is a serious problem for dog owners as well as the veterinary clinicians.

A Dutch study from 2019 investigated co-carriage of ESBL producing bacteria in humans and dogs and humans and cats belonging to the same households and found co-carriage between humans and the dogs in the same household. None were found in the case of humans and cats in the same households (van den Bunt, et al., 2020). This raises a concern of how important animal to human transference of ESBL producing bacteria is and how it may impact the spread of the ESBL genes.

A Swedish study from 2015 showed that ESBL producing *E. coli* could be isolated from raw diets made for dogs (Nilsson, 2015). Commercially available raw food diets for dogs were sampled in and around Uppsala in Sweden and 39 samples from 8 different brands were taken. 23% of the samples contained ESBL producing *E. coli* (*see figure 2*). These products were intended for dogs, not humans. But since one can assume that many of these products are being handled in kitchens where food for human consumption is prepared as well, there is a risk of cross-contamination in households where raw food dog diets are being served. Thus, not only contact with companion dogs but also contact with such raw canine diets may serve as a source of ESBL producing bacteria (Nilsson, 2015).

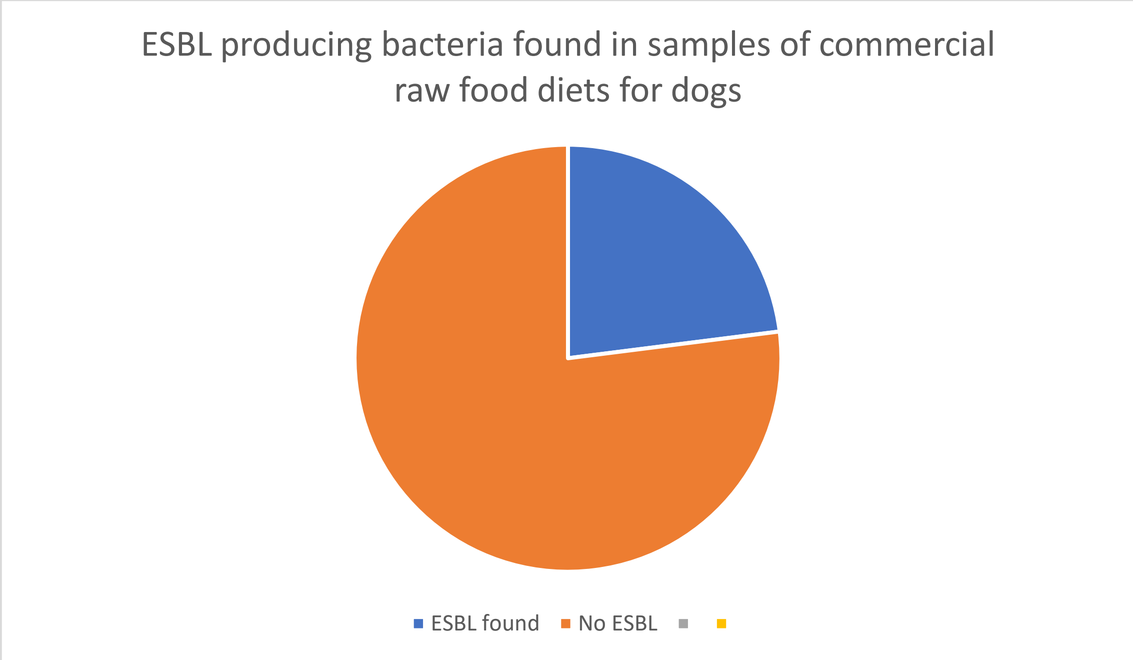

Figure 2. Proportion of commercial raw food diets for dogs containing ESLB producing bacteria.

In the case of Swedish horses, the emergence of ESBL producing bacteria has been a cause of concern and the SVARM project called for closer monitoring of the situation in 2010 (National Veterinary Institute (SVA), 2010). When horses were first tested for ceftiofur resistance within the SVARM research in 2002, no such resistance was over the limit of Minimum Inhibition Concentration (MIC-value) and in the clinical isolates investigated within the SVARM reports from 2002 to 2007, no ESBL was confirmed even though a few isolates with ceftiofur resistance were detected throughout these years. The most common diagnostic submissions were isolates of *Streptococcus zooepidemicus* from the respiratory tract and *E. coli* from the genital tract of mares. The exclusion of repeated diagnostic samples from the same individual or stable could not be guaranteed and the samples were not equally distributed throughout the country. In 2008, one clinical isolate from the genital tract of a mare was confirmed as a ESBL producing *E. coli*. Clinical isolates in horses from 2015 also came from the genital tract of mares and it should be noted that cephalosporins are, in some instances, used in stud farms to treat certain gynaecological conditions in mares according to the SVARM report in 2008 (National Veterinary Institute (SVA), 2008) which may cause a selection pressure of ESBL producing pathogens among these horses. The issue was further investigated in 2010 when 200 diagnostic submissions from the genital tract of mares were screened for ESBL producing bacteria. 8 of the isolates obtained were confirmed to be ESBL producing *Klebsiella pneumoniae* and 4 of those *Klebsiella pneumoniae* isolates came from the same stud farm. Additionally, 431 faecal samples from 17 different equine stud farms (147 samples) and horses sampled upon arrival to animal hospitals (284 samples) were taken during the same investigation. 6 of these samples had ESBL producing *E. coli*. In conclusion, 8 out of 200 from the mare's genital tracts and 6 out of 431 faecal samples carried ESBL producing bacteria (National Veterinary Institute (SVA), 2010), and these are indeed quite low occurrences in this set of samples.

All the ESBL producing bacteria found in horses between 2008 and 2011 were also multi-resistant and besides resistance to extended spectrum cephalosporins they were also not susceptible to gentamicin, trimethoprim or sulphonamides. 63% of those bacteria were resistant to tetracyclines as well. Such high occurrence of multiresistance among the ESBL producing isolates varied among dogs and cats tested during the same time period (National Veterinary Institute (SVA), 2010).

In 2011 we saw another high proportion of clinical isolates from horses test positive for ESBL production when the SVARM report reported 14 isolates from horse's wounds, female genital tract, eyes and form unknown origins were tested and all of them had ESBL producing *E. coli* or *Enterobacter* spp (Swedish National Veterinary Institute (SVA), 2011). An increase in ESBL producing isolates has thus been seen since 2008 and perhaps it could be explained by increased awareness from the veterinary clinicians, or it might be a true increase of ESBL producing bacteria within the horse populations in Sweden. In 2016 there were 18 clinical isolates confirmed to be ESBL producing, which until then was the highest number of ESBL producing bacteria confirmed from horses in a year (Public Health Agency of Sweden; National Veterinary Institute (SVA), 2016) and since then the number of confirmed ESBL producing isolates has increased. In 2017 the number had risen to 31 isolates confirmed to produce ESBL (Public Health Agency of Sweden;National Veterinary Institute (SVA), 2017). It should be noted that around this time, SVA started offering ESBL screening free of charge and this increase in isolates submitted for ESBL confirmation may very well be affected by this. Since around this time however, numbers of ESBL producing bacteria in clinical isolates from horses in Sweden has kept at approximately 20 per year since 2018 until 2022.

Leaving the annual number of ESBL producing bacteria in Swedish horses behind, a concern with ESBL producing bacteria is their multiresistance. In a somewhat worrisome study from 2019 ESBL producing *Enterobacteriaceae* from Swedish horses were investigated for colistin resistance (Börjesson, et al., 2020). Colistin, also called Polymyxin E, has been widely used in the animal production industry for a long time as it is an effective treatment for infections caused by *Enterobacteriaceae* spp. Colistin has recently gained importance as a last- resort treatment for multiresistant bacterial infections in humans (Kempf, et al., 2016), and therefore colistin resistant ESBL producers should not be taken lightly. An investigation in 2019 of a sample collected from a horse in 2018 showed ESBL producing *E. coli* with the colistin resistance gene. This became the first finding of a ESBL producing *E. coli* carrying the colistin resistance gene in animals in Sweden (Publich Health Agency of Sweden; National Veterinary Institute (SVA), 2019). The study from 2019 was therefore initiated and 56 bacterial isolates of the following species were included: *E. coli*, *Enterobacter cloacae*, *Klebsiella oxytoca* and a species of *Citrobacter* taken from horse's wounds, surgical wounds, uterus, urine, and abscesses, amongst others. 30 out of these 56 ESBL producing isolates also had the colistin resistance gene (Börjesson, et al., 2020). However, none of the isolates had a MIC value above the cut-off value, meaning although the bacteria carried the gene for resistance, they would be susceptible to high enough levels of colistin. The colistin resistance gene in ESBL producing isolates from horses in this study was therefore common. Colistin is not used in horses in Sweden, although it is used within the Swedish pig industry. Polymyxin B is, however, occasionally used as a topical treatment for companion animals in Sweden (Börjesson, et al., 2020), and in a British study from 2021 the authors were of the opinion that even if it is not currently known if use of Polymyxin B in the treatment of SIRS (systemic inflammatory response syndrome) in horses in the UK contributes to colistin resistance, colistin should be used with prudence and care to minimize the global impact these antibiotics (Isgren, 2022). The same might be said for the use of Polymyxin B in companion animals here in Sweden.

The detection of colistin resistant ESBL producing bacteria in Swedish animals should be regarded as a severe finding due to the importance of colistin in the human healthcare. Especially since $ESBL_{CARBA}$ is seen in increasingly high numbers in Swedish hospital settings (Public Health Agency of Sweden; National Veterinary

Institute, 2022), and colistin is one of the antibiotics that remains an option for treatment in these patients. With bacteria such as these, we are left with little to no treatment options, and one cannot disregard the serious impact they could have if they were to spread. In Swedish horses there would be no authorised treatment option left if these ESBL producing colistin resistant bacteria were to cause infection today (Börjesson, et al., 2020).

3.6 ESBL producing bacteria spreading between humans and animals.

As we see the increase of ESBL producing bacteria on a global scale we must work to understand every aspect of the properties that facilitates their spread. It is paramount that we have a thorough understanding of these resistant bacteria to successfully prevent their spread and prevent and treat infections caused by them. In conclusion, the emergence of ESBL producing bacteria in the world warrants the question of transference between animals and humans. The risks associated with ESBL producing bacteria spreading between humans and animals are not only due to the risks associated with the increasingly difficult to treat infections that they can cause, but also since individuals, humans, or animals, carrying these bacterial genes could provide as reservoirs for their continued spread and development (EFSA Panel of Biological Hazards (BIOHAZ), 2011). As stated earlier, ESBL producing bacteria have been increasing in human healthcare. While the Covid-19 pandemic brought us a decrease in the occurrence of these bacteria, we are now looking at an increase once more (*see figures 3 and 4.)* (Public Health Agency of Sweden; National Veterinary Institute, 2022).

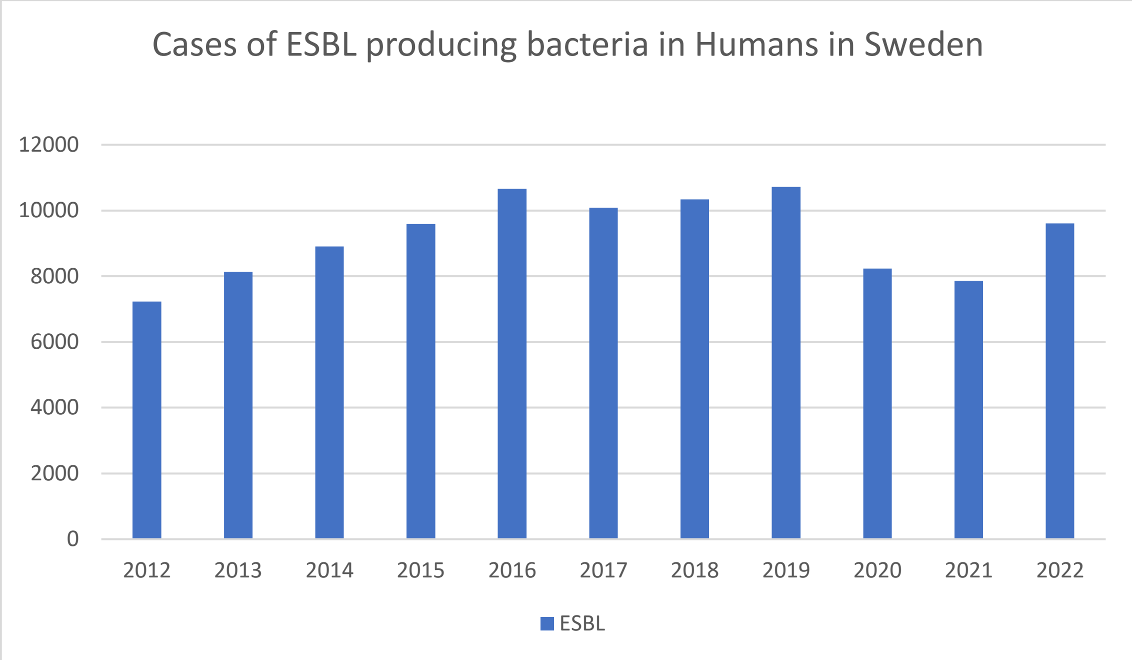

Figure 3. Number of cases of ESBL producing bacteria reported in humans in SWEDRES-SVARM reports 2012-2022.

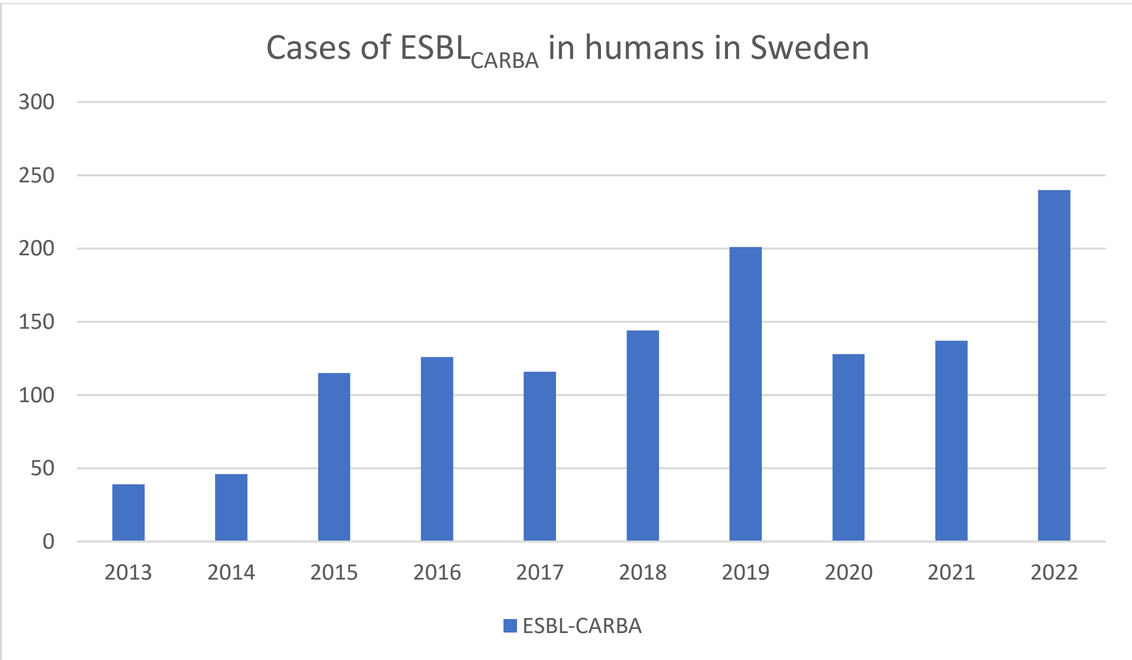

Figure 4. Cases of $ESBL_{CARBA}$ reported in humans in SWEDRES-SVARM reports 2013-2022.

ESBL genes are, like many antibiotic resistance genes, mostly located on plasmids (Paterson & Bonomo, 2005; Bennet, 2008). Plasmids are types of mobile genetic elements able to transfer a group of genes from one bacterial cell to another. This allows for horizontal gene transfer, a phenomenon that enables bacteria to acquire new genetic material, effectively adding to their genome instead of just replicating it. Thus, bacterial genomes can change in two ways; mutations within their own genetic material, and acquisition of new genetic material via routes such as plasmids. This renders them extremely efficient in adapting to the environmental challenges they encounter. The exchange of certain adaptive traits via plasmids allows them to respond to the specific demands of their surroundings and ESBL production in response to extended spectrum cephalosporin use is an example of that. Abilities such as horizontal gene transfer are a major factor in the rise of antibiotic resistance (Bennet, 2008).

The Gram-negative family *Enterobacteriaceae* contains many of the most commonly occurring ESBL producers, namely *E. coli* and *Klebsiella pneumoniae*. *E. coli* is also used as indicator bacteria in the Swedish SVARM and SWEDRES monitor programmes and in similar programmes in the EU (European Food Safety Authority (EFSA);European Centre for Disease Prevention and Control (ECDC), 2023). *E. coli* and *Klebsiella pneumoniae* are the most common ESBL producing bacteria in Swedish hospitals, and they cause disease in humans such as urinary tract infection and septicaemia (Åhrén, 2017; Public Health Agency of Sweden; National Veterinary Institute, 2022). Furthermore, *E. coli* are commensal gut bacteria and as such, *E. coli* is found in the gastrointestinal tract of both humans and food producing animals (Public Health Agency of Sweden; National Veterinary Institute, 2021). This is an important factor in the spread of ESBL genes since these commensal bacteria are so widespread in the environment and have the ability to exchange ESBL genes through horizontal transfer. All environments in which ESBL producing bacteria are allowed to fraternize with other bacteria are therefore opportunities for them to transfer their resistance genes to other bacteria of the same species, or indeed, to a different bacterial species as well.

ESBL producing bacteria are reported more frequently in humans than in animals. In humans, ESBL producing *Enterobacteriaceae* is the most common type of notifiable antibiotic resistance within the SWEDRES-SVARM project (Public Health Agency of Sweden; National Veterinary Institute, 2022) while we see only a few percentages of most production animals carrying the transferable ESBL genes in Sweden (except for

Swedish broilers where almost half of the tested broilers carried the genes a few years ago) (Swedish National Veterinary Institute (SVA), 2011; Public Health Agency of Sweden; National Veterinary Institute, 2022). In 2012 7225 cases of ESBL producing *Enterobacteriaceae* were reported in humans in Sweden, it was an increase with almost 30% since the previous year (National Veterinary Institute; Swedish Institute for Communicable Disease Control., 2012). The Covid pandemic at the start of 2019 caused the numbers of cases with ESBL producing *Enterobacteriaceae* to drop significantly and this was a trend seen in almost all forms of antibiotic resistance monitored during the pandemic years *(see figures 3 and 4)* (Public Health Agency of Sweden; National Veterinary Institute, 2022). From 2020 to 2021, there was a 4% decrease of ESBL cases while some of the pandemic measures were still in place (Public Health Agency of Sweden; National Veterinary Institute, 2021). In 2022 we saw the numbers increase again when 9611 cases were reported, an increase of 22% since 2021. Cases of $ESBL_{CARBA}$ producing bacteria in humans have also been on the rise since the pandemic. These bacteria have not yet been found in Swedish domestic animals (Public Health Agency of Sweden; National Veterinary Institute, 2022).

Even though numbers of cases are relatively small in the animal sector, there have been studies showing carriage of ESBL producing bacteria on different types of animal products meant for human consumption. A report created by SVA, the National Food Agency and the Swedish Institute for Communicable Disease Control examined the incidence of ESBL producing bacteria, namely *E. coli*, in animal products on the Swedish market in 2011 (Livsmedelsverket, 2011). 0-8% of the imported beef sampled, 2-13% of the imported pork and a concerningly high, although varying 15-95% of broiler meat samples were confirmed to contain ESBL producing *E. coli*. The highest percentage of ESBL carriage in broiler meat, 95% of the samples, came from South America. The European broiler meat was shown to have a very high percentage of these bacteria also, as 61% of those samples were positive. Although ESBL producing *E. coli* was found more frequently in these imported products, 44% of 100 samples of Swedish broiler meat tested in the same study also carried the ESBL producing bacteria in 2011. Thus, ESBL producing *E. coli* was found in all types of animal products tested in this study, including the domestic products.

The risk of these animal products transferring ESBL producing *E.* coli to humans might be small according to a study done in 2016 on the dissemination of ESBL from food and food producing animals in Sweden (Börjesson, et al., 2016). The study examined to what degree ESBL producing *E. coli* found in humans, food and food producing animals had overlapping genes and found that the overlap was limited. Thus, the study did not find any significant evidence of ESBL producing *E. coli* spreading from animals or animal products to humans. The most common route of dissemination in humans was human to human contact. Furthermore, the study concluded that travels to Asian and African countries was a greater factor for human carriage of ESBL producing *E. coli* than contact with food or food producing animals carrying the bacteria. Though the results of this study showed limited dissemination, there is a possibility of spread between humans and animals, nonetheless. Although only 2 out of 103 isolates from humans were identified as possibly linked with poultry the study found that broiler meat can act as a dissemination route to humans and furthermore, that poultry can serve as a reservoir for the ESBL genes. The author of the Swedish study also mentions a German study published in 2014 that found a greater overlap between the *E. coli* genes in animal and human populations (Valentin, et al., 2014) but acknowledges that it is difficult to directly compare the two studies due to differences in methodologies. Contrasting this Swedish study mentioned above, a Dutch study with samples from store bought chicken meat and humans taken between 2008 and 2009 in the southern parts of the Netherlands showed a greater correlation between the ESBL producing *E. coli* found in broiler chickens and humans. They found an overlap both in the mobile elements, i.e., plasmids, as well as chromosomal traits. This suggests that there is both plasmid transference between chicken sourced strains of *E. coli* to human intestinal *E. coli* strains and direct bacterial transference in the Dutch food chain (Kluytmans, et al., 2013). As mentioned earlier, these studies might not be directly comparable due to differences in methodologies, but these two German and Dutch studies shows that a transference of ESBL producing bacteria between humans and animal species might be relevant.

4. Conclusions

In this literature review, I aimed to establish what the status of ESBL producing bacteria looks like in Sweden with emphasis on food producing animals and pets. I have found that ESBL producing bacteria is not very common amongst companion animals, horses or food producing animals in Sweden with the exception of broilers in the 2010s. The Swedish broiler industry did experience a steep increase in ESBL producing bacteria in 2010 but since then numbers have dwindled and in the most recent SWEDRES-SVARM report, ESBL producing bacteria was at low levels in all animals monitored. A study of the ESBL occurrence in Swedish broilers showed that the ESBL producing bacteria was largely an imported problem, and this was in line with the absence of cephalosporin antibiotic use in the Swedish broiler industry. Another study issued by the SVA, the National Food Agency and the Swedish Institute for Communicable Disease Control showed that ESBL producing bacteria was rather common on imported meat samples. Antimicrobial resistance at large is a well monitored threat in Sweden, and we do have several programmes and cross-sectional cooperations in place for the monitoring and prevention of this threat. Even so, the rise of ESBL producing bacteria in the Swedish broiler production shows that the issue of antimicrobial resistance is only an import away.

I believe the infected imported broiler stock suggests that even though Sweden may have a good status regarding ESBL producing bacteria and generally, a strong biosecurity in our animal industries, the products, or animals that we import have the ability to damage that good status. It therefore highlights the need for international cooperations because imports and exports absolutely pose a risk of spreading bacteria with multiresistance genes. Countries such as Sweden, with a generally good status of antimicrobial resistance and biosecurity in the food chain should be careful when importing animals and animal products from countries with higher occurrences of resistance and Swedish customers should be encouraged to buy from Swedish rather than imported animal products. The occurrence of ESBL producing bacteria in Swedish broilers after imports is a good example of why a consumer should perhaps prefer the Swedish products because it supports the Swedish work against antimicrobial resistance in a way that certain imported animal products cannot.

There have in fact been campaigning from the food industry to increase the incitive of Swedish people to buy Swedish animal products and in my opinion, there is a general consensus among the public today that Swedish animal products are a more conscious choice than imported ones. The awareness of antimicrobial resistance planted in the Swedish society in the last century and early 2000s is indeed helpful in promoting the responsible use of antimicrobial drugs in both humans and animal healthcare. And the SWEDRES-SVARM reports seem vigilant and thorough in their research and monitoring. However, there is yet no continuous monitoring of indicator bacteria in companion animals and the data from companion animals rely mostly on the submission of clinical isolates to estimate the occurrence of ESBL producing bacteria in these animals in Sweden. A Dutch study found a co-carriage of ESBL producing bacteria between human and dogs living in the same household and a Swedish study found commercial raw feed diets from dogs to carry ESBL producing bacteria. Even though ESBL producers in clinical isolates from companion remains low, the potential of co-carriage between humans and pets indicates that a closer monitoring of the situation in companion animals is warranted. Furthermore, the detection of ESBL producing bacteria carrying the colistin resistance gene in horses is a serious finding that requires closer, more active monitoring and perhaps a more comprehensive study than the one done on 56 bacterial isolates from horses in 2019.

In conclusion, this literature review shows that Sweden has low levels of ESBL producing bacteria in the animal sectors thus far and that the occurring problems regarding ESBL producing bacteria in the broiler industry and meat products have been mostly imported. The low occurrence is a success and Sweden does indeed seem capable of handling the challenges presented in the broiler industry thus far, but research presented in this paper also shows that ESBL producing bacteria, like any multiresistant bacteria, is a serious threat to antibiotics. Antibiotics are unmistakeably one of modern society's greatest and most important inventions and we must protect them as much as we can from bacterial resistance. The rise of ESBL producing bacteria is an unavoidable threat that must be dealt with, however low the case numbers might be, they must be taken seriously and handled with vigilance.

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